REMARKS

Claims 1-6 and 10-25 are active. Claim 24 finds support in the original claims and on page 29, line 11 which describes a polymer thickness of less than 10 nm. Claim 25 finds support at the top of page 29 of the specification. Accordingly, the Applicants do not believe that any new matter has been introduced. Favorable consideration of this amendment and allowance of the application are respectfully requested.

Aspects of the Invention to Consider

Unlike denaturing prior art methods, the invention provides a sensor, such as a biochip, having improved sensitivity in which the attached proteins preserve their active sites. The more active sites available for binding, the more sensitive the sensor.

This technical problem is solved by the present invention by the electropolymerization conditions (the amount of current applied and the time of application) as defined in claim 1 which make it possible to obtain a sensor having a pyrrole film with a thickness less than or equal to 10 nm as shown in Table 3.

Table 3

Blocks	Synthesis time (ms)	Charge (μC)	Charge ($\mu C/mm^2$)	Thickness (nm)	IF (AU)
A	**250**	10	**25**	**5**	**90**
B	**500**	15	**37**	**7.2**	**102**
C	1000	20	50	10	160
D	2000	37	90	18	140
E	4000	58	142	28	90
F	8000	115	287	56	80
G	16 000	205	512	102	30

The thickness of the pyrrole polymer, as shown at [0172-0173], not only plays a key role in the capacity for attachment and recognition of proteins—including hindered proteins—but also influences the biological response of the sensor. No prior art has

described or suggested that the thickness of the polymer may have an influence on the biological response of a biosensor (specification, page 30, last paragraph).

In addition to the positive effects on biological response of a biosensor, the inventors have demonstrated that a pyrrole polymer with a thickness less than or equal to 10 nm is necessary to obtain accurate results when sensors produced according to the invention are implemented by optical techniques, such as plasmon resonance. The prior art does not suggest selection of a pyrrole polymer film for a biosensor having a thickness less than or equal to 10 nm (which as about the diameter of the immobilized protein molecule).

Domb—Continuation-in-Part// Response to Examiner's Argument

The Applicants previously urged that the Domb CIP did not trace support back to its priority document PCT/IL00/00807[1]. In the response to the Applicants' argument on page 11 of the Official Action ("OA") the Examiner points to Example 2 "Synthesis of Pyrrole Analogs" starting at the bottom of page 17 of PCT '807. However, this section does not expressly describe the pyrrole-protein coupling compound, and mixing such a coupling compound with a second solution of uncoupled pyrrole monomer as required by the present claims.

The Examiner also refers to lines 23-28 of page 18 of PCT '807. Line 23 indicates "heparin is conjugated", however, heparin is not a protein or peptide, but a highly-sulfated glycosaminoglycan. Lines 1-6 on page 19 are also mentioned; these lines refer to "direct esterification to aminopropyl pyrrole" of "hydroxy containing bioactive molecules" using carbodiimides. While peptides may contain hydroxy side-chains, this section is silent with respect to production of a protein-pyrrole coupling compound and mixing such a coupling

[1] Page 11 of the Official Action refers to PCT '806, which the Applicants presume refers to PCT '807.

compound with a second solution of uncoupled pyrrole monomer as required by the present claims.

Furthermore, while [0019] and [0027] of the latter Domb CIP publication is relied upon (OA, top of page 3) for teaching "coating of electropolymerized pyrrole polymers to a conductive support", the Office has not pointed out support for this in Domb '807, which is directed to coating implantable devices (see Title), and does not suggest selecting and applying a biofilm having a thickness of 10 nm or less to a biosensor substrate. The detailed objectives of Domb '807 on pages 9-10 does not mention electropolymerization under the conditions specified by claim 1 or to a biosensor support as required by claim 2.

Should a future rejection rely upon Domb, the Applicants respectfully request that for simplicity that the Examiner point out support for such a rejection in Domb '807 to avoid the issue of basing such a rejection on new matter which is not prior art.

Accordingly, the rejections below based on Domb cannot be sustained, because the sections relied upon by the Examiner are not prior art, and, in any event do not suggest or provide a reasonable expectation of success for the superior biosensors provided by the invention.

Rejection—35 U.S.C. §103(a)

Claims 1-3, 6 and 10-23 were rejected under 35 U.S.C. §103(a) as being unpatentable over Livache, et al., Biosens. Bioelec. 13:629, in view of Domb, U.S. 2006/0013850 and Guedon, et al., Anal. Chem. 72: 6003. The prior art does not render the invention obvious because it does not suggest or provide a reasonable expectation of success for it.

As indicated in the Official Action, Livache does not disclose **pyrrole monomers coupled to peptides/proteins** via use of activated pyrrole.

Domb is a continuation-in-part which was filed after the effective filing date of this pending application. New matter added to Domb after November 30, 2000—the filing date of the Domb priority application PCT/IL00/00807—is not prior art as discussed above.

Review of the Domb priority document PCT '807 shows that it refers to polymeric coatings containing oxidized polypyrrole derivatives with anionic peptides and proteins (page 21, Example 3 and claims 21-22). Thus, Domb PCT '807 does not disclose the coupling of an activated pyrrole to a protein to form a protein-pyrrole coupling compound in which the coupling implements a covalent bond between the activated pyrrole and the protein as required by the present invention.

On the contrary, Domb PCT '807 discloses an electrostatic complex between the oxidized polypyrrole derivatives and the anionic peptides and proteins. Assuming *arguendo* that Domb teaches a pyrrole covalently bound to a peptide or protein, it does not expressly describe production of a pyrrole-protein coupling compound, mixing such a coupling compound with a second solution of uncoupled pyrrole monomer, and the specific electropolymerization steps as required by the present claims.

In addition, Domb PCT '807 indicates "the polypyrrole coating is oxidized by applying an oxidizing potential" (Example 3, page 21), which clearly means that the ionic bond between the oxidized polypyrrole derivatives and the anionic peptides and proteins takes place after the electropolymerization step.

Moreover, the optimal thickness of the spot given in Guedon which is close to 11 nm cannot anticipate the optimal thickness required in the present application which is less than or equal to 10 nm (see e.g., claims 15, 19 and 23).

Guedon also is directed to DNA sensors and methods for making them, is does not suggest or provide a reasonable expectation of success for producing the protein sensors of the invention. The prior art does not suggest modifying the prior art DNA sensors to produce

protein sensors and the prior art does not provide a reasonable expectation of success for obtaining a useful protein sensor. For example, methods for making DNA sensors do not face the problems such as the possibility of denaturation and the need to preserve of protein active sites and involve molecules (nucleic acids) having different sizes and chemical characteristics. Nothing in the prior art would have suggested to one of ordinary skill in the art that the teaching of DNA sensors using oligonucleotides can be modified and applied to proteins sensors which must face problems linked to preservation of protein active sites and protein size. On the other hand, the claimed method involves the attachment of a protein to a conductive support in order to produce useful protein-based sensor such as those used on biochips. The present invention also provides a method for obtaining a sensor with an improved sensitivity and in which the attached proteins are not denatured and preserve protein active sites. None of these features are contemplated by the prior art which is directed to a different chemical class of molecule—nucleic acids.

The inventors have solved the particular technical problems associated with proteins by the particular electropolymerization conditions (the amount of current applied over the time applied) described in claim 1. This procedure makes it possible to obtain a sensor having a pyrrole film with a thickness of less than or equal to 10 nm as shown in Table 3. Indeed, as indicated in the application at [0172] and [0173], the thickness of the pyrrole polymer not only plays a key role in the capacity for attachment and recognition of proteins, including hindered proteins, but also has an influence on the biological response of the sensor. In addition, the inventors have shown that a pyrrole polymer with a thickness less than or equal to 10 nm is necessary to obtain accurate results when sensors produced according to the invention are implemented using optical techniques including plasmon resonance. The prior art does not provide a reasonable expectation of success for these

properties of the invention, nor suggest the underlying structure providing these properties. Accordingly, this rejection should now be withdrawn.

Rejection—35 U.S.C. §103(a)

Claim 4 was rejected under 35 U.S.C. §103(a) as being unpatentable Livache, et al., Biosens. Bioelec. 13:629, in view of Domb, U.S. 2006/0013850 and Guedon, et al., Anal. Chem. 72: 6003, and further in view of Caillat, et al., U.S. Patent No. 6,803,228. The primary references have been addressed above and do not suggest or provide a reasonable expectation of success for the invention. Caillat was cited as teaching a pyrrole polymer functionalized with N-hydroxysuccinimide and maleimide, but does not suggest the other aspects of the invention. Accordingly, this rejection may be withdrawn for the reasons discussed above.

Rejection—35 U.S.C. §103(a)

Claims 1-3, 6 and 10-21 were rejected under 35 U.S.C. §103(a) as being unpatentable over Livache, et al., Biosens. Bioelec. 13:629, Domb, U.S. 2006/0013850, Guedon, et al., Anal. Chem. 72: 6003, and Caillat, et al., U.S. Patent No. 6,803,228, as applied to claim 4 above, and further in view of Bianchi, et al., U.S. 2003/0207400.

The primary references have been addressed above and do not suggest or provide a reasonable expectation of success for the invention. Bianchi was cited as teaching various linkers to functionalize pyrrole with thiol, maleimide or amino groups. However, Bianchi does not suggested or provide a reasonable expectation of success for the invention described in independent claim 1. Accordingly, this rejection may be withdrawn for the reasons discussed above.

Rejection—35 U.S.C. §103(a)

Claims 1-3 and 6-9 were rejected under 35 U.S.C. §103(a) as being unpatentable over Livache, et al., Anal. Biochem. 255:188, in view of Livache, et al., Biosens. Bioelec. 13:629, [Domb, U.S. 2006/0013850,] and Guedon, et al., Anal. Chem. 72: 6003[2].

Livache, Anal. Biochem., concerns polypyrrole DNA chips. Even if this document suggested that copolymerization of many "biological agents", it does not specifically suggest peptides or polypeptides and is silent about the problems associated with these types of molecules. Livache concerns DNA chips and biological molecules like RNA. In addition, it discloses that the optimal thickness for the polymer film is 20 nm (see page 192, right col., 2nd paragraph), not the less than or equal to 10 mm required by claims 15, 19 and 23.

As already explained above, nothing in Livache, Biosens. Bioelec., and/or Guedon would have suggested to one of ordinary skill in the art a method for making a protein sensor with an improved sensitivity and in which the attached proteins present preserved active sites as required by independent claim 1. The method of claim 1 requires coupling an activated pyrrole with a protein and submitting the protein to an electropolymerization under particular conditions (current and time) which enables the production of a sensor having a pyrrole film with a thickness of less than or equal to 10 nm. Accordingly, this rejection should be withdrawn since the prior art does not suggest the method of claim 1 or provide a reasonable expectation of success for the sensitive protein-based sensors provided by this method.

Livache et al.

Livache was previously cited as prior art and as disclosing coupling a pyrrolyl residue to a dT10 oliognucleotide linker which is coupled to a synthetic peptide. In Livache it is stated that the pyrrole ODN and pyrrole peptides were prepared by coupling a pyrrolyl

[2] The body of this rejection (see page 10 of the OA) relies on Domb, U.S. 2006/0013850. The Applicants respectfully request that the Examiner clearly indicate this in the initial statement of the rejection.

residue on an ODN or a synthetic peptides through a dT10 oligonucleotide linker (page 630, §1). A dT10 oligonucleotide is a large molecule and corresponds to ten residues of deoxythymidine (dT). The coupling of an ODN on a peptide as proposed in Livache can make it possible to improve (1) the peptide purification thanks to a "standard" solubility (an HPLC column in neutral conditions instead of in acid conditions can thus be used) and (2) its detection (UV at 260 nm). Nevertheless, this document does not disclose or suggest coupling a peptide or protein directly to an activated pyrrole.

Livache being unconcerned with proteins, provides no indication concerning any experimental procedure showing the possibility to apply the process involving the dT10 oligonucleotide to peptides and proteins. Livache explicitly shows the possibility of building an ODN pyrrole using an oligonucleotide synthesizer (page 2916, col. 2, §2). Nevertheless, such a system is not applicable to protein since protein chemistry is different from the nucleoside chemistry. The information given in Livache is not sufficient to enable a person skilled in the art to apply the dT10 oligonucleotide linker process to a protein, because of the fundamental difference between the chemistry of peptides and nucleotides and the lack of any guidance in Livache for such a process.

On the other hand, the present application requires coupling a peptide to an activated pyrrole monomer in distinction to Livache which suggests using a dT10 linker between a pyrrolyl residue and a peptide. Accordingly, the Applicants respectfully submit that Livache would not apply to the present claims.

CONCLUSION

In view of the above amendments and remarks, the Applicants respectfully submit that this application is ready for allowance. Early notification of such is earnestly requested.

Respectfully submitted,

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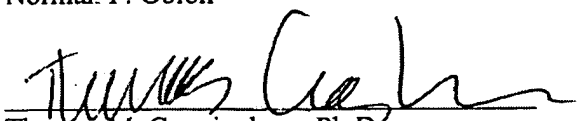

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